CLAIMS

- 1. A bioactive polypeptide, MF3, with a primary structure depicted in SEQ ID NO:1, an active fragment of MF3, or any functional derivative of MF3, said polypeptide, active fragment or functional derivative being capable of effecting a resistance of a plant against microbial diseases and/or against attack of plant parasites.
- 2. An isolated DNA sequence depicted in SEQ ID:2, or fragment thereof, encoding a
 functionally active MF3 or its active fragment according to claim 1, wherein said
 DNA fragment may contain degenerate codons.
 - 3. A method of acquiring resistance of a plant against microbes and/or plant parasites by introducing the bioactive polypeptide MF3, or an active fragment, or a functional derivative thereof into plants mechanically or by means of carrier molecules.
 - 4. The method according to claim 3, wherein the carrier is chitosan.
 - 5. A vector comprising the DNA according to claim 2.

20

- 6. The method of generating a transgenic plant or plant cell culture comprising a vector according to claim 5, wherein the plant cells express the polypeptide encoded by the DNA.
- 7. A host cell stably transformed or transected with a vector of claim 5.
 - 8. A plant protectant composition comprising isolated components of claim 1.
- 9. The active fragment of MF3 according to claim 1, wherein the amino acidsequence consists of SEQ ID:3 or SEQ ID:4.
 - 10. A method of isolating and purifying the polypeptide of claim 1 from bacterial cells expressing said polypeptide, the method comprising the steps:

- a) cultivating a microbial producer strain and extracting cells with a buffer solution at an elevated temperature;
- b) precipitating a crude MF3 polypeptide at low temperature with a precipitant;
- c) fractionating re-dissolved precipitate by an anion exchange chromatography
- 5 column and collecting fractions with anti-microbial or anti-insect activities;
 - d) performing polyacrylamide gel electrophoresis of the polypeptide fractions with anti-microbial, anti-nematode, or anti-insect activities;
 - e) recovering the protein eluted from the gel of step d.

10

15

20

SUMMARY OF THE INVENTION

The object of the invention is a protein, termed MF3, or a functional derivative thereof with a novel structure that surprisingly can induce multiple resistance in plants toward a variety of viral and microbial infections and against pests. The invention also concerns an isolated DNA sequence encoding MF3 protein, as such, or as a part of any DNA sequence, or a fragment thereof, or DNA sequences which have degenerate codons with respect to the DNA sequence defined above. The invention also concerns a method of isolating and purifying the protein MF3 from bacterial cells expressing the said protein and its use as a plant protectant with or without carrier agent Furthermore, the invention concerns a method of obtaining transgenic plants expressing said protein. A further object of the invention is the use of the protein, or of compositions containing the same, as a plant protectant, biopesticide for inducing resistance of plants to viral, microbial phytopathogens and pests.

REFERENCES

5

10

15

20

25

30

Pietrzak, M., Shillito, R.D., Hohn, T., Potrykus, I. Expression in plants of two bacterial antibiotic resistance genes after protoplast transformation with a new plant expression vector. √ Nucleic Acids Res., 1986, v.14, pp.5857-5868.

Bevan, M. Binary *Agrobacterium* vectors for plant transformation. √ Nucleic Acids Res., 1984, v.12, pp.8711-8721.

Van Haute E., Joos, H., Maes, S., Warren, G., Van Montagu M., Schell, J. - Intergeneric transferband exchange recombination of restriction fragment cloned in pBR322:a novel strategy for reversed genetics of the Ti plasmids of Agrobacterium tumefaciens. EMBO J., 1983, v.2, pp.411-418.

Baulcombe D. 1994. Novel strategies for engineering virus resistance in plants. Current Opinion in Biotechnology., 5, 117-124.

Bradford M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 276-287.

Christophe Breton, Hichem Chellil, Majida Kabbaj-Benmansour, Eric Carnazzi, René Seyer, Sylvie Phalipou, Denis Morin, Thierry Durroux, Hans Zingg, Claude Barberis, and Bernard Mouillac. 2001.Direct Identification of Human Oxytocin Receptor-binding Domains Using a Photoactivatable Cyclic Peptide Antagonist .Comparison with the human V1 a vasopression receptor. J. Biol. Chem., Vol. 276, Issue 29, 26931-26941, July 20, 2001

Cion R.A. The manual of determinative microbiology. OGIZ-SELHOZGIZ. Moscow. 1948. p. 484 (In Russian).

de Barjac H. and A. Bonnefoi. 1967. A classification of strains of Bacillus thuringiensis Berliner with a key to their differentiation. J Invertebr. Pathol. 11, 335-347.

de Barjac H. and A.Bonnefoi. 1967. Classification des souches de Bacillus thuringiensis. C R Acad Sci (Paris) 264, 1811-1813.

Golishin N.M. Fungicide in agriculture, Moscow, "Kolos" 1982.pp. 20-66

Jackman P.J.H. Microbial systematics based on electrophoretic whole-cell protein patterns. Methods in microbiology. Academic Press, London. Ed. by R.R.Colwell and Grigorova R.. 1987. Vol.19. pp. 210-224.

Krieg A. 1968. A taxonomic study of Bacillus thuringiensis Berliner. J. Invertebr. Pathol. 12, 366-378.

Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

Latterell F.M., Marchetti M.A., Grove B.R. Co-ordination of effort to establish an international system for race identification in *Pyricularia oryzae*. The Rice Blast Disease. Baltimore, Maryland: The Johns Hopkins Press: 1964. pp. 257-268.

Sambrook J., Fritsch E.F. and Maniatis T., 1989. Molecular cloning. A Laboratory Manual 2nd ed. Cold Spring Harbor Laboratory Press.

Schroder K, Zuber P., Willimsky G., Wagner B. and Marahiel MA.1993. Mapping of the Bacillus subtilis csp B gene and cloning of its homologs in thermophilic, mesophilic and psychrotrophic bacilli. Gene, 136, 277-280.

Smith N.R., R.E.Gordon and F.E.Clark. 1952. Aerobic sporeforming bacteria. Agr.Monogr. 16 U S Dept. Agr, pp. 1-148.

The shorter Bergey's manual of determinative bacteriology // The Williams and Wilkins Company, Baltimore. Ed. by Holt. 1980. P.495.

10